Attorney Docket No. 9536-3 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Vance et al. Confirmation No.: 6465
Application Serial No.: 10/623,930 Group Art Unit: 1638
Filed: July 21, 2003 Examiner: V. Kumar
For: *Compositions and Methods for the Modulation of Gene Expression In Plants*

Declaration of Lewis H. Bowman, Ph.D. Pursuant to 37 C.F.R. § 1.132

I, Lewis H. Bowman, do hereby declare and say as follows:

1. I am a named inventor under United States Application No. 10/623,930 ("the '930 application") and of the subject matter claimed therein.

2. I have a Ph.D. in Biology from the University of Virginia. I am an Associate Professor of Biological Sciences at the University of South Carolina, Columbia, SC. I am involved in research in the area of gene silencing and small RNA biology in plants and have authored or co-authored more than 25 publications related to this area. A *curriculum vitae* is attached herewith at Tab B.

3. I have read and understand the publications by Krol et al. (J. Biol. Chem. 279: 42230-42239 (2004)), Alvarez et al. (Plant Cell 18: 1134-1151 (2006)) and Lee et al. (EMBO J. 20: 4663-4670 (2002)), which were cited by the Examiner in connection with the '930 application.

4. The focus of Krol et al. is comparison of predicted miRNA precursor structures as compared to those determined experimentally. Krol et al. experimentally determined the structures of ten human miRNA precursors in solution and showed that the experimentally determined structures of 8 of 10 of these differ from that predicted by the computer program MFOLD. However, it is noteworthy that the percentage of correctly predicted base pairs for the ten precursors is 88.4%, which is higher than that usually obtained when predicting the structures of other RNAs (73-83%) (Krol et al., page 42237, column 1). Thus, compared to most RNAs, it is relatively easier to predict the structure of the miRNA precursors. Furthermore, the majority of the incorrect predictions were found

to occur in the terminal loop (Krol et al., Fig. 2). Since the present invention only describes altering the miRNA and miRNA* sequences, such incorrect predictions in the terminal loop region are not relevant to the '930 application. In fact, overall the results presented in Krol et al. indicate that MFOLD does an excellent job predicting the structure of the miRNA precursor, especially in the miRNA-miRNA* region (*Id.*). Furthermore, analysis of the ten precursors described in Krol et al. as well as the predicted miRNA precursors (*Id.*) indicates that there is considerable flexibility in the structure of the miRNA-miRNA* region as long as this region is extensively base paired as described in the present specification.

Thus, contrary to the assertions of the Action, Krol et al. shows that the structures of the miRNA precursors are predictable most of the time (base pairs correctly predicted 88.4% of the time) and when one is specifically discussing miRNA-miRNA* region, the percent correctly predicted is even higher. Therefore, contrary to the assertions in the Office Action, one of skill in the art would view Krol et al. as supportive of the '930 invention working as described in the specification.

5. Alvarez et al. shows that expression of endogenous miRNA genes from a tissue specific promoter or a nontissue specific promoter results in significant loss of the mRNAs targeted by the miRNA in the appropriate tissues and further shows the expected phenotypes associated with the reduced expression of these genes. Of particular interest in Alvarez et al. is the construction of artificial miRNAs which when introduced into plants were properly processed, incorporated into RISC, and successfully reduced the level of the targeted mRNAs causing phenotypes consistent with the reduced levels of target genes. The artificial mRNAs were constructed by altering Arabidopsis 164a and the 164b miRNA and miRNA* sequences as described in the '930 application. Thus, as the Office Action points out, they took into consideration the bulges present in the endogenous miRNA as one is directed to do in the '930 application. Alvarez et al. also showed that Arabidopsis miRNAs, including the artificial miRNA, can effectively regulate cross-species targets. This paper clearly demonstrates that the methodology described in the '930 application can be used to construct artificial miRNAs that effectively down regulate their intended targets.

6. Lee et al. shows that miRNA are processed from their precursors in a two step process in Hela (human) cells and that some precursors contain more than one miRNA sequence. The first step occurs in the nucleus and excises the pre-miRNA from the precursor (*See*, Lee et al., page 4665, both columns and 4667, first column, second full paragraph). In subsequent work by Lee et al., this excision step was shown to be due to the activity of Drosha (*Nature* 425: 415-419 (2003)). The second step, which generates the mature miRNA occurs in the cytoplasm and is mediated by the enzyme, Dicer (*See*, Lee et al., page 4665, both columns and 4667, first column, second full paragraph). Furthermore, Lee et al. shows that the signals for cleaving the pre-miRNA from the larger precursor are located in or very close to the pre-miRNA itself.

These steps outlined in Lee et al. for the processing of the clustered miRNA genes are the same steps as those used in the processing of a single miRNA precursor. (See, for example, Lee et al., page 4667, first column, second full paragraph; Mica et al., page 2601, second column second full paragraph; and Kurihara and Watanabe, *Proc. Natl. Acad. Sci. U S A.* 101, 12753-12758 (2004) generally, but at least page 12756, last paragraph) (copy enclosed)). Thus, the excision of multiple pre-miRNAs from a polycistronic precursor containing more than one pre-miRNA sequence is no different than the excision of one. The processing of plant miRNA precursors is similar to that in mammals with some minor differences, for instance, DICER LIKE 1 is likely responsible for both processing steps (Kurihara and Watanabe, *Proc. Natl. Acad. Sci. U S A.* 101, 12753-12758 (2004)).

Accordingly, an investigator in this field of research would reasonably understand that miRNA precursors useable with the presently claimed invention would include those that occur singly and those that occur as clusters. In further support of this assertion, Nui et al. (*Nat Biotechnol.* 24(11):1420-8 (2006)) were able to produce two different artificial miRNAs in a coordinated manner using a dimeric gene that they constructed. The dimeric construct contained the two individual artificial miRNA constructs ligated together and under control of the same promoter. A copy of the Nui et al. publication was submitted with the Vance Declaration and the previous response on January 3, 2007.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001

of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Lewis H. Bowman

Lewis H. Bowman, Ph.D.

8/20/07

Date

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Date: August 20, 2007

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Tab B

CURRICULUM VITAE

NAME	POSITION TITLE
Lewis H. Bowman	Associate Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR	FIELD OF STUDY
Duke University, Durham, NC	B.A.	1971	Psychology/Chemistry
University of Virginia, Charlottesville, VA	Ph.D.	1979	Molecular and Developmental Biology

A. Positions and Honors.

1979-1982	Postdoctoral Fellow, Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO (with D. Schlessinger, rRNA processing studies)
1982-1988	Assistant Professor, Department of Biological Sciences, University of South Carolina, Columbia, SC
1988-present	Associate Professor, Department of Biological Sciences, University of South Carolina, Columbia, SC

B. Selected peer-reviewed publications.

Bowman, L.H. and Emerson, C.P., Jr. (1977) Post-transcriptional regulation of ribosome accumulation during myoblast differentiation. *Cell* 10:587-595.

Bowman, L.H. and Emerson, C.P., Jr. (1980) Formation and stability of cytoplasmic mRNAs during myoblast differentiation: Pulse-chase and density labeling analyses. *Devel. Biol.* 80:146-166.

Bowman, L.H., Rabin, B., and Schlessinger, D. (1981) Multiple ribosomal RNA cleavage pathways in mammalian cells. *Nucleic Acid. Res.* 9:4951-4966.

Bowman, L.H., Goldman, W.E., Goldberg, G.I., Herbert, M.B., and Schlessinger, D. (1983) Location of the initial cleavage sites in mouse pre-rRNA. *Mol. Cell. Biol.* 3:1501-1510.

Vance, V.B., Thompson, E.A., and Bowman, L.H. (1985) Transfection of mouse ribosomal DNA into rat cells: Faithful transcription and processing. *Nucleic Acids Res.* 13:7499-7513.

Bowman, L.H. (1987) rDNA transcription and pre-rRNA processing during the differentiation of a mouse myoblast cell line. *Devel. Biol.* 119:152-163.

Agrawal, M.G. and Bowman, L.H. (1987) Transcriptional and translational regulation of ribosomal protein accumulation during mouse myoblast differentiation. *J. Biol. Chem.* 262:4868-4875.

Bowman, L.H. (1987) The synthesis of ribosomal proteins S16 and L32 are not autogenously regulated during mouse myoblast differentiation. *Mol. Cell. Biol.* 7:4464-4471.

Hammond, M.L. and Bowman, L.H. (1988) Insulin stimulates the translation of ribosomal proteins and the transcription of rDNA in mouse myoblasts. *J. Biol. Chem.* 263:17785-17791.

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Kim, K., Lecordier, A., and Bowman, L.H. (1995) Both nuclear and mitochondrial cytochrome c oxidase mRNA levels increase dramatically during mouse postnatal development. *Biochem. J.* 306:353-358.

Marathe, R., Smith, T.H., Anandalakshmi, R., Bowman, L.H., Fagard, M., Vaucheret, H. and Vance, V.B. (2000) Plant viral suppressors of post-transcriptional silencing do not suppress transcriptional silencing. *Plant Journal* 22: 51-59.

Thames, E.L., Newton, D.A., Black, S.A., and Bowman, L.H. (2000). Role of mRNA stability and translation in the expression cytochrome c oxidase during mouse myoblast differentiation: instability of the mRNA for the liver isoform of subunit VIa. *Biochem. J.* 351: 133-142.

Anandalakshmi, R., Marathe, R., Ge, X., Herr, J.M., Mallory, A., Mau, C., Pruss, G., Bowman, L.H., and Vance, V.B. (2000) A calmodulin-related protein from tobacco suppresses post-transcriptional gene silencing. *Science* 290: 142-144.

Mallory, A.C., Ely, L., Smith, T.H., Marathe, R., Anandalakshmi, R., Fagard, M., Vaucheret, H., Pruss, G., Bowman, L., Vance, V.B. (2001) HC-Pro suppression of transgene silencing eliminates the small RNAs but not transgene methylation or the mobile signal. *Plant Cell* 13: 571-83.

Mallory, A., Parks, G., Endres, M., Baulcombe, D., Bowman, L.H., Pruss, G.J., and Vance V.B. (2002) The amplicon-plus system for high-level expression of transgenes. *Nat Biotechnol* 20, 622-5.

Mallory, A.C., Reinhart B.J., Bartel, D.B., Vance, V.B. and Bowman, L.H. (2002) A viral suppressor of RNA silencing differentially regulates the accumulation of short interfering RNAs and microRNAs in tobacco. *Proc Natl Acad Sci USA* 99, 15228-15233.

Mallory, A.C., Mlotshwa, S., Bowman, L.H. and Vance, V.B (2003) The capacity of transgenic tobacco to send a systemic RNA silencing signal depends on the nature of the inducing transgene. Plant J. 35, 82-92.

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Mlotshwa, S., Schauer, S., Smith, T. H., Mallory, A., Herr, J.M., Roth, B., Merchant, D., Ray, A., Bowman, L. and Vance, V. (2005) Ectopic DICER-LIKE1 expression in P1/HC-Pro Arabidopsis rescues phenotypic anomalies but not defects in microRNA and S\silencing pathways. Plant Cell 17, 2873-85.

Johnson, C., Bowman, L., Adai, A.T., Vance, V., Sundaresan, V. (2007) CSRDB: a small RNA integrated database and browser resource for cereals. Nucleic Acids Res. 35, D829-D833.

C. Research Support

6445532-0517482 07/01/05-06/30/08 $1,203,620.
NSF/Plant Genome
Small RNAs in Rice and Maize, Lewis Bowman, Principal Investigator, Co-Principal Investigators, Vance Bowman, Sundaresan, Bullard-Dillard
The major goals of this research are to clone and sequence small RNA populations in rice and maize, identify microRNAs and examine their biogenesis.
Role: Principal Investigator

9/1/07-8/31/09 $300,000
NSF
The Role of Arabidopsis RAV2 in Viral Suppression of RNA Silencing. Lewis Bowman, PI and Vicki Vance CO-PI.
The major goal of this research is determine the role of putative transcription factor, RAV2, in the suppression of RNA silencing mediated by HC-Pro.
Role: Principal Investigator

D. Patents

Issued US Patents
Co-inventor, "Compositions and Methods for Manipulation of Gene Expression in Plants. Filed in 2002, Pending.

Patents Pending
Co-inventor, "An Endogenous Regulator of RNA Silencing in Plants", filed June 2005.